REMARKS

Claims 1-10 were previously cancelled and new claims 11-33 were added. Claims 11-14 and 22-30 are hereby cancelled and claims 15 and 14-19 are hereby amended and new claims 34-37 have been added. Claim 15 has been amended and placed in independent form. Claims 19-27 have been amended to depend from claim 15 rather than canceled claim 11.

Therefore, claims 15-21 and 31-37 are currently pending.

The Invention

The inventors have discovered that mucosal administration of a mixture of a surface antigen of a virus and a second vaccine antigen is effective in generating an enhanced immune response for the prevention or treatment of an infection by either the virus from which the surface antigen is derived, or the agent from which the second vaccine antigen is derived.

Objections listed in the Office Action of June 4, 2002

In the Office Action issued on June 4, 2002, the Examiner objected to Figure 5 of the drawings because the text within the drawing is in the Spanish language.

Applicants herewith have provided Figure 5 with the English annotation as required by the Examiner. Therefore, the objection to the figure is moot and must be withdrawn.

Objection to Alleged New Matter

In the Office Action the amended "Summary of the Invention" was objected to under 35 U.S.C. 132, because according to the Examiner, the amendment added new material not supported by the specification.

The Summary of the Invention has been amended herein to delete the reference to the surface antigen component of the vaccine as "a virus-like particle (VLP)."

Rejections under 35 U.S.C. §112, second paragraph

In the Office Action claims 11-33 were rejected under §112, second paragraph for the recitation of "...VLP and a vaccine antigen, which provides an adjuvant enhancing effect on either the VLP, the vaccine antigen or each other." According to the Examiner, it cannot be determined whether a physical linkage or fusion is intended by this phrase, or whether the antigens are within the same proximity.

Applicants have cancelled claims 11-14 and 22-30, and have amended claims 15 and 19. Claim 15 as amended recites "...A vaccine formulation suitable for mucosal administration, comprising: a mixture of a first vaccine antigen which is Hepatitis B virus surface antigen (HBsAg) and a second vaccine antigen which is a non-living vaccine antigen comprising an antigen of a viral nucleocapsid, wherein said second vaccine antigen has an adjuvant effect on HBsAg, and wherein said first and second vaccine antigens are each present up to about 1 mg."

The noun "mixture" has a clear and definite meaning: According to the Webster's New Twentieth Century Dictionary, (Second Ed., 1983), at page 1153 (attached as Exhibit 1), the noun "mixture" means "the state of being mixed." Further, this dictionary states that the noun "mixture" as used in chemistry is defined as "a substance containing two or more elements: distinguished from *compound* in that the constituents are not in fixed proportions and do not lose their individual characteristics." Thus, a "mixture" is distinguished from a chemical compound with fixed proportions of constituents.

Therefore, Applicants assert that the meaning of the term "mixture" is clear and definite. The claimed vaccines are mixtures of a first vaccine antigen which is a surface antigen from a virus, and a second vaccine antigen, wherein the surface antigen enhances the immune response to the second vaccine antigen. As explained above, the pending claims reciting a "mixture" of vaccine antigens cannot be interpreted to encompass embodiments wherein the first and second vaccine antigens are fused or chemically linked. Therefore this rejection should be withdrawn.

At page 3 of the Office Action of June 4, 2002, claim 19 is rejected as allegedly unclear in stating that the formulation comprising HBsAg and the vaccine antigen "comprises a single antigen." Claim 19 depends from claim 15, which has been amended to separate the two descriptive clauses with a semi-colon and now recites: "...A vaccine formulation comprising: (a) a mixture of a first vaccine antigen which is Hepatitis B virus surface antigen (HBsAg), and (b) a second vaccine antigen which is a non-living vaccine antigen comprising an antigen of a viral nucleocapsid;..." Applicants therefore maintain that claim 19 is clear and definite. Therefore, this rejection should be withdrawn.

At page 4 of the Office Action the Examiner rejected claims 28-30 under 35 U.S.C. §112, second paragraph as allegedly vague and indefinite in stating that the immune response is enhanced, since according to the Examiner, it cannot be determined in what way the immune response is enhanced or what type of immune response is enhanced. Applicants have cancelled claims 28-30, therefore this rejection under 35 U.S.C. §112, second paragraph is moot and must be withdrawn.

For all the above recited reasons, Applicants maintain that pending claims 15-21 and 31-37 as amended are clear and definite and the rejections under 35 U.S.C. §112, second paragraph should be withdrawn, which action is respectfully requested.

Rejections under 35 U.S.C. §112, first paragraph

At page 4, the Examiner rejected claims 11-33 under 35 U.S.C. §112, first paragraph for reasons of record, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one of skill in the art that the inventor(s) had possession of the invention at the time the application was filed.

Specifically, the Examiner alleges that the nature of the mixture and the mixing process is unclear. Applicants point to the ample description in Examples 2-5 of the specification wherein vaccine mixtures of HBcAg and HBsAg; HBcAg, HBsAg and VLPs of Human Papilloma Virus 16; HBsAg and VLPs of Human Papilloma Virus 16; and also HCV nucleocapsid and HBsAg are described.

Thus, the claimed mixtures of a first vaccine antigen which is a surface antigen of a virus and a second vaccine antigen which is a non-living vaccine antigen, are indeed described in the specification in such a way as to convey to one of skill in the art that the inventor had possession of the claimed invention at the time of filing of the application. Therefore, Applicants assert that the rejection of claims 11-33 under 35 U.S.C. §112, first paragraph should be withdrawn.

At page 5, the Examiner rejected claim 11 under 35 U.S.C. §112, first paragraph for the recitation of "a virus-like particle (VLP) comprising a surface antigen from a virus." Claim 11

has been canceled and claim 15 *et seq.* has been amended and does not refer to a virus-like particle (VLP). Similarly, dependent claims 16-18 do not recite any requirement for a VLP. Therefore this rejection should be withdrawn.

Claims 22-27 were also rejected under 35 U.S.C. §112, first paragraph as allegedly not being adequately described and also for claiming new matter. Claims 22-27 have been cancelled hereby. Therefore, the rejection of these claims as new matter and under 35 U.S.C. §112, first paragraph is moot and must be withdrawn.

At pages 5-9, the Examiner rejected claims 15-19, 22-27 and 31-33 under 35 U.S.C. §112, first paragraph for reasons of record, as allegedly failing to enable one of ordinary skill in the art to which it pertains, or is most nearly connected, to make and/or use the invention.

According to the Examiner, it cannot be determined whether the claims are directed to treating and preventing both hepatitis B and the infectious disease caused by the agent from which the vaccine antigen is derived (such as HPV or HCV) or just hepatitis B (HBV).

Applicants assert that one of ordinary skill in the art would clearly understand that the vaccines of the present invention are directed to prevention and therapy of hepatitis B disease through an anti-hepatitis B immune response (Abstract) and also prevention and therapy of the disease caused by the agent from which the second vaccine antigen is derived (specification page 7, lines 8-10). Thus Applicants maintain that it is clear that the claims are directed to treating and preventing both hepatitis B and the infectious disease caused by the agent from which the

vaccine antigen is derived (such as HPV or HCV). Therefore, Applicants respectfully assert that this rejection should be withdrawn.

In addition, the Examiner again questions whether the HBsAg is encapsulated within the various VLPs, or whether the VLPs and HBsAg are formed into a chimeric molecule or are simply provided in a mixture. However, the Examiner goes on to acknowledge the teachings in the specification of the mixing of antigens, and to the co-administration of the various antigens (in the Examples at page 7-11).

Claims 15-27 and 31-37 do not recite any requirement that any of the vaccine antigens of the claimed mixture is encapsulated, or that one of the vaccine antigens be in the form of a VLP. Therefore this rejection is moot and must be withdrawn.

According to the Examiner, the specification fails to teach how to identify a mixture, fusion or complex of HBsAg and any viral nucleocapsid that would satisfy the intended vaccine function. The Examiner comments that the working examples in the specification are limited to administering combinations of antigens in mice and monitoring antibody response. Further, the Examiner offers the opinion that those of skill would have no way to predict how long the elevated antibody responses lasted after administration of the vaccines.

The Examiner summarizes by stating that it would require undue experimentation to practice the claims due to the alleged ambiguity of the claims; the scope of the claims to preventing and treating any infection with the vaccine composition; the lack of guidance for making chimeric/encapsulated molecules; the lack of guidance as to the CTL responses or the

persistence of the antibody responses; the lack of an appropriate animal model or data demonstrating prevention or treatment by vaccine administration; and the lack of predictability in the vaccine art.

Applicants deny the Examiner's assertion that it would require undue experimentation to practice the claimed invention. A declaration under 37 C.F.R. §1.132 of Dr. Jules César Aguilar Rubido, a co-inventor named in the present application, is submitted herewith. In the declaration, Dr. Rubido states at paragraph 7 that the results of administration of a mixture of HBsAg and HBcAg show an improved cellular response as compared with HBsAg alone. Further Dr. Rubido states that these results are consistent with the higher IgG2a responses for HBsAg mixed with HBcAg compared with the control HBsAg alone.

At paragraph 8, Dr. Rubido states that the HBsAg plus HBcAg mixture also induces a better lymphoproliferative response than the control HBsAg alone and that the strong lymphoproliferative response correlates with a better course of infection by the HBV.

Moreover, at paragraph 9, Dr. Rubido states that the mixture of HBsAg and HBcAg potentiates the immune response against both antigens, evidencing the synergistic interaction between both antigens. Dr. Rubio states that similar results are also seen other HBsAg plus nucleocapsid antigens (paragraph 10) as compared with the control HBsAg alone (paragraph 11).

At paragraphs 12 and 13, Dr. Rubido states that the experiments reported in Example 2 of Exhibit 1 show an enhancing capacity and new properties after nasal administration of the new formulations of HBsAg and virus-like particles (VLPs) as compared with the VLP alone in a transgenic mouse model. Immune tolerance against HBsAg in transgenic mice was shown to be

abrogated by administration of the claimed HBsAg plus HBcAg formulation and correlated with the disappearance of HBsAg from the blood. Again, Dr. Rubido states at paragraph 13 citing table 1, this is in contrast to the result obtained with the commercial HBsAg vaccine, Engrix B adsorbed onto alum salts or alone, administered intraperitoneally.

As stated by Dr. Rubido in the attached declaration at paragraphs 14-16, the chimpanzee is not the only suitable animal model for HBV infection in humans. The immunized mouse model and transgenic mouse model are sufficient to support the initiation of human trials of the claimed mixtures and therefore are *prima facie* adequate models for HBV infection humans.

As demonstrated by the examples disclosed in the present specification and as explained by Dr. Rubido in the attached declaration, one of ordinary skill in the art of vaccine research and development would clearly understand that the results disclosed would have led to a reasonable expectation of success of the claimed vaccine mixtures in inducing an enhanced immune response to the antigen components of the mixture as compared to the single antigens alone.

For all these reasons, Applicants assert that it would not require undue experimentation to practice the claimed invention and that the pending claims are fully supported in their broadest scope by the specification as filed which teaches that a specific immune response to both the first vaccine antigen and to the second vaccine antigen are generated in the mouse model and that the vaccine antigen exert a synergistic effect on the immune response to each other. Because such data are generally accepted by those of skill in the art as a reasonable basis for initiation of clinical trials, applicants assert that the burden of proof of demonstrating a reasonable expectation of success has been met.

Therefore, Applicants respectfully assert that the rejection of claims 15-19 and 22 under 35 U.S.C. §112, first paragraph should be withdrawn.

Rejection under 35 U.S.C. §102(a)

Initially, in the Office Action of June 4, 2002, Examiner rejected claims 11-14, 20, 21, 28-30 under 35 U.S.C. §102(a) as clearly anticipated by Balmelli et al. (J. Virol. 72(10): 8220-8229. However, this rejection was not explained in the Office Action and was subsequently withdrawn in the Interview Summary issued June 18, 2002. Therefore this rejection will not be considered further.

Rejection under 35 U.S.C. 103(a)

At page 9 the Examiner rejected claims 11-14, 20, 21, 28-30 under 35 U.S.C. §103(a) as allegedly unpatentable over Lowy et al., US Patent No. 5,618,536 and Rose et al., US Patent No. 6,153,201.

Claims 11-14 and 28-30 have been canceled. Therefore, the rejection of claims 11-14 and 28-30 under 35 U.S.C. §103(a) as allegedly unpatentable over Lowy et al. (supra) and Rose et al. are moot and should be withdrawn, which action is earnestly solicited. Furthermore, claims 20 and 21 have been amended to depend from claim 15. Thus, claims 20 and 21 recite a vaccine formulation comprising a surface antigen of Hepatitis B virus (HBsAg) and a second vaccine antigen which is an antigen of a viral nucleocapsid. Nowhere in Lowy et al. or Rose et al. is there any suggestion of the use of a surface antigen of Hepatitis B virus (HBsAg) in a mixture

with an antigen of a viral nucleocapsid. Therefore, claims 20 and 21 are not obvious over Lowy et al. and Rose et al. and this rejection of claims 20 and 21 should also be withdrawn.

If the Examiner has any questions or comments relating to the present application, he or she is respectfully invited to contact Applicants' attorney at the telephone number set forth below. Applicants submit that the application is now in condition for examination on the merits.

Respectfully submitted,

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161925_1

EDITED VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification

Please delete Figure 5 and replace with the attached Figure 5 with wording in English.

Please delete the first paragraph of the amended "Summary of the Invention" at page 5 and replace with the following:

- - The present invention provides a vaccine formulation suitable for mucosal administration, the vaccine includes a mixture of [a virus-like particle (VLP) comprising] a surface antigen from a virus, and a non-living vaccine antigen, the surface antigen having an adjuvant effect on said vaccine antigen. Each vaccine dose includes up to about 1 milligram each of the surface antigen and vaccine antigen. The vaccine formulation may include one or more of the following: a preservative, a stabilizer and a second vaccine antigen. - -

At page 6, please delete the paragraph extending from line 13 to line 16 and replace with the following:

- - -It is possible to obtain a wider spectrum of immune response generated by HBcAg regarded as an important antigen *per se* in anti-HBV [VHB] protection. Furthermore, the IgG seric levels anti-HBsAg reached by mucosal inoculation is as intense as the one obtained with the systemic inoculation in alum. - -

In the claims:

Please cancel claims 11-14 and 28-30. Also, please amend claims 15-21 and 31-33 and add new claims 34-37 as follows: (All the pending claims 15-27 and 31-37 are recited below for the Examiner's convenience).

15. (Amended) [The] A vaccine formulation [according to claim 11, wherein the surface antigen] suitable for mucosal administration, comprising:

(a) a mixture of a first vaccine antigen which is Hepatitis B virus surface antigen (HBsAg), and [the]

(b) a second vaccine antigen which is a non-living vaccine antigen comprising an antigen of a viral nucleocapsid,

wherein said second vaccine antigen has an adjuvant effect on HBsAg, and wherein said first and second vaccine antigens are each present up to about 1 mg.

16. (Amended) The vaccine formulation according to claim 15, wherein the viral nucleocapsid is [a virus-like particle comprising] the nucleocapsid antigen of Hepatitis B virus.

17. (Amended) The vaccine formulation according to claim 15, wherein the viral nucleocapsid is [a virus-like particle comprising] the nucleocapsid antigen of Human Papilloma-virus.

18. (Amended) The vaccine formulation according to claim 15, wherein the viral nucleocapsid is [a virus-like particle comprising] the nucleocapsid antigen of Hepatitis C virus.

19. (Twice amended) The vaccine formulation according to claim [11] 15, wherein the [surface antigen is Hepatitis B virus surface antigen (HBsAg); and the] second vaccine antigen comprises a single antigen or a mixture of different antigens that are immuno-enhanced by HBsAg.

20. The vaccine formulation according to claim [11] 15, wherein the vaccine formulation is suitable for administration as a solid, liquid or spray.

21. (Amended) The vaccine formulation according to claim [11] 15, wherein the vaccine formulation is suitable for nasal administration.

22. (Amended) The vaccine formulation according to claim [11] 15, wherein the vaccine formulation is suitable for use as a therapeutic vaccine against Hepatitis B virus (HBV) infection.

23. (Amended) The vaccine formulation according to claim [11] 15, wherein the vaccine formulation is suitable for use as a preventive vaccine against Hepatitis B virus (HBV) infection.

24. The vaccine formulation according to claim [11] 15, wherein the vaccine formulation is suitable for use as a preventive vaccine against Hepatitis C virus (HCV) infection.

25. The vaccine formulation according to claim [11] 15, wherein the vaccine formulation is suitable for use as a preventive vaccine against Human Papilloma virus (HPV) infection.

26. The vaccine formulation according to claim [11] 15, wherein the vaccine formulation is suitable for use as a therapeutic vaccine against Hepatitis C virus (HCV) infection.

27. The vaccine formulation according to claim [11] 15, wherein the vaccine formulation is suitable for use as a therapeutic vaccine against Human Papilloma virus (HPV) infection.

31. (Amended) The vaccine formulation according to claim 19, wherein the second vaccine antigen comprises the core antigen of Hepatitis B virus.

32. (Amended) The vaccine formulation according to claim 19, wherein the second vaccine antigen comprises the nucleocapsid antigen of Hepatitis C virus.

33. (Amended) The vaccine formulation according to claim 19, wherein the second vaccine antigen comprises the nucleocapsid antigen of Human Papilloma-virus.

34. (New) The vaccine formulation according to claim 15, wherein said second vaccine antigen has an adjuvant effect on said surface antigen.

35. (New) The vaccine formulation according to claim 15, further comprising a preservative.

36. (New) The vaccine formulation according to claim 15, further comprising a stabilizer.

37. (New) The vaccine formulation according to claim 15, further comprising a third vaccine antigen.

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family *Cracidæ* and genus *Mitu* or *Mitua*, of which it is the type; the galeated curassow.

Mit'ū·a, *n.* 1. a genus of insects, order *Coleoptera*.
2. a genus of birds, type of the family *Cracidæ*.

mīt'y, *a.* having or abounding in mites.

mitz'vāh (mits'), *n.*; *pl.* **mitz'vōth,** in Judaism, (a) a commandment or precept, as in the Bible or from a rabbi; (b) an act fulfilling such a command or the spirit of such commands; as, an act of charity is a *mitzvah*: also spelled *mitsvah*.

mix, *v.t.*; mixed *or* mixt (mikst), *pt., pp.*; mixing, *ppr.* [ME. *mixen*; AS. *miscian*, to mix; akin to O.H.G. *miskan*, Russ. *mieshatĭ*, W. *mysgu*, L. *miscere*, Gr. *misgein*, *mignynai*, to mix; Sans. *micra*, mixed.]
1. to unite or blend promiscuously into a mass or compound; as, to *mix* flour and salt; to *mix* wines; also, to make by putting ingredients together; as, he *mixed* a cake.
2. to join; to associate; to unite with in company.
Ephraim, he hath *mixed* himself among the people. —Hos. vii. 8.
3. to cause to join or associate.
You *mix* your sadness with some fear. —Shak.
4. to crossbreed.
Syn.—blend, compound, confuse, join, mingle, unite.
to mix up; (a) to mix thoroughly; to mingle together; (b) to confuse; to cause confusion in; also, to mistake for another (with *with*); (c) to involve or implicate (*in* some matter).

mix, *v.i.* 1. to be mixed or capable of being mixed; to be blended; to mingle; as, oil and water will not *mix* readily.
2. to associate or get along together; as, to *mix* in society.

mix, *n.* 1. a mixing or being mixed.
2. a muddle; state of confusion.
3. a prepared blend of various ingredients; mixture.
4. a beverage, usually carbonated, as soda or ginger ale, for mixing with alcoholic liquor.

mix'ā·ble, *a.* capable of being mixed.

mixed (mikst), *a.* 1. joined or mingled in a single mass or compound; blended.
2. made up of different or incongruous parts, elements, classes, races, etc.
3. consisting of or involving both sexes; as, a *mixed* class, *mixed* company.
4. confused; muddled.
5. in phonetics, central: said of vowels.
mixed marriage; marriage between persons of different religions or races.
mixed number; in mathematics, a number expressed by a whole number and a fraction (e.g., $2^1/_4$).
mixed voices; in music, male and female voices combined.

mix'ed·ly, *adv.* in a mixed manner; promiscuously.

mix'en, *n.* a compost heap.

mix'ēr, *n.* 1. one who or that which mixes.
2. a kitchen apparatus, operated by hand or by an electric motor, used to mix dough, beat eggs, whip cream, etc.
3. in mechanics, a valve in which air and fuel are mixed in the proper proportions to obtain efficient combustion.
4. a person with reference to his degree of social adaptability; as, he is a good (or bad) *mixer*; also, a party or gathering for the purpose of furthering social relationships. [Colloq.]
5. in metallurgy, a tank in which molten pig iron may be stored after it has been removed from a blast furnace and before it is placed in a converter or in an open-hearth furnace.

mix'īte, *n.* [named after A. *Mixa*, a Bohemian commissioner of mines.] a mineral of a bluish green color, an arsenate of copper and bismuth, found first in Bohemia and afterward in Utah: it occurs in capillary crystals.

mix'ō-, [Gr. *mixo-*, from *mignynai*, to mix.] a combining form meaning *mixed*, as in *mixo*gamy, *Mixo*dectidæ.

mix"ō·bär·bar'iç, *a.* [Gr. *mixobarbaros*, half barbarous; *mixo-*, from *mignynai*, to mix, and *barbaros*, barbarous.] not altogether barbaric; partly civilized.

Mix·ō·deç'tēṣ, *n.* [*mixo-*, and Gr. *dēktēs*, a biter, from *daknein*, to bite.] the typical genus of Eocene mammals of the family *Mixodectidæ*.

Mix·ō·deç'ti·dae, *n.pl.* [*Mixodectes* and *-idæ*.] a family of prosimian Eocene mammals characterized by very large incisors and simple premolars in the lower jaw.

mix·og'ā·mous, *a.* [*mixo-*, and Gr. *gamos*, marriage.] in ichthyology, characterized by mixogamy.

mix·og'ā·my, *n.* in ichthyology, the coming together of males and females in unequal numbers at spawning time, males being in excess.

mix·ō·lyd'i·ăn mōde, [Gr. *mixolydios*, half-Lydian; *mixo-*, mixed, and *Lydios*, Lydian.]
1. in ancient Greek music, one of the four principal modes.
2. in medieval music, an authentic ecclesiastical mode, the seventh in number, based on G, with D dominant and C mediant.

mixt, *v.* alternative past tense and past participle of *mix*.

Mix'teç, *n.* an Indian, one of the inhabitants of early Mexico, living in certain parts of Oaxaca and Guerrero (near the present city of Acapulco); also, the speech of these Indians, a branch of the Zapotec language.

Mix·te'çō, Mix·te'çā (mēs-), *n.* same as *Mixtec*.

mix'ti·form, *a.* [L. *mixtus*, pp. of *miscere*, to mix, and *forma*, form.] characterized by mixture in form; made of mixed materials. [Rare.]

mix·ti·lin'ē·ăl, *a.* [L. *mixtus*, mixed, and *linea*, line.] containing a mixture of lines, as straight, curved, etc.; as, a *mixtilineal* angle (i. e., an angle contained by a straight line and a curve).

mix·ti·lin'ē·ăr, *a.* mixtilineal.

mix'tion (miks'chun), *n.* [L. *mixtio* (*-onis*), a mixture, mixing.]
1. mixture; promiscuous assemblage. [Obs.]
2. a mixture of mastic, amber, etc., used as a medium or mordant for affixing gold leaf.

mix'tūre, *n.* [Late ME.; OFr.; L. *mixtura*, a mixing.]
1. the act of mixing or state of being mixed.
2. a mass or compound consisting of different ingredients blended without order.
3. something mixed, as a cloth made of differently colored thread.
4. in pharmacy, a liquid medicine which contains insoluble matter suspended in some viscid substance.
5. in chemistry, a substance containing two or more elements: distinguished from *compound* in that the constituents are not in fixed proportions and do not lose their individual characteristics.
6. in music, an organ stop, of a shrill and piercing quality, consisting of two or more ranks of pipes: called also *furniture stop*.
Syn.—compound, composition, combination.

mix'ty-max'ty, *a.* promiscuously mingled. [Scot.]

mix'-up, *n.* 1. a mingling of things or of people in disorder; a confusion; tangle.
2. a fight. [Colloq.]

miz'māze, *n.* [reduplication of *maze*.] a maze or labyrinth. [Obs.]

miz'päh, *n.* [Heb.] literally, a watchtower; the name of several places in ancient Palestine, especially applied to the heap of stones gathered in Mt. Gilead by Jacob and his brethren as a remembrance of the covenant made with Laban (Gen. xxxi. 49): in modern usage the word signifies a parting salutation, suggested by Laban's prayer—"The Lord watch between me and thee, when we are absent from one another."

miz'zen miz'en, *a.* [Late ME. *meseyn*; OFr. *misaine*; It. *mezzana*, fem. of *mezzano*, middle, from L. *medianus*, from *medius*, middle.] of the mizzenmast.

miz'zen, miz'en, *n.* 1. a fore-and-aft sail set on the mizzenmast.
2. a mizzenmast.

miz'zen·mâst, miz'en·mâst, *n.* the mast supporting the mizzen; the mast that stands nearest to the stern in a ship with two or three masts.

miz'zle, *v.i.* and *v.t.*; mizzled(-zld), *pt., pp.*; mizzling, *ppr.* [ME. *miselen*, freq. of *misten*, to mist.] to rain in very fine drops; to drizzle. [Obs. or Dial.]

miz'zle, *n.* fine rain; mist; drizzle. [Obs. or Dial.]

miz'zle, *v.i.* to depart suddenly; to leave unceremoniously. [Brit. Dial.]

miz'zle, *v.t.* and *v.i.* to confuse; to entangle the mind of; to become confused or entangled, as with drink. [Dial.]

miz'zly, *a.*; *comp.* mizzlier; *superl.* mizzliest; misty: said of the atmosphere.

Mn, in chemistry, manganese.

mnē·mon'iç (nē-), *a.* [Gr. *mnēmonikos*, pertaining to the memory, from *mnēmōn* (*-onis*), mindful, from *mnāsthai*, to remember.]
1. assisting or intended to assist the memory.
2. pertaining to mnemonics.

mnē·mon'iç·ăl, *a.* mnemonic.

mnē·mō·ni'ciăn (-nish'un), *n.* an expert in a system of mnemonics.

mnē·mon'içs, *n.pl.* [Gr. *mnēmonika*, mnemonics, neut. pl. of *mnēmonikos*, mnemonic.]
1. [*construed as sing.*] the science or art of improving the memory, as by the use of certain formulas.
2. formulas or other aids to help in remembering.

mnē'mō·nist, *n.* a mnemonician.

Mnē·mos'y·nē, *n.* [Gr. *mnēmosynē*, memory.] in Greek mythology, the goddess of memory and mother (by Zeus) of the Muses.

mnē'mō·teçh·ny, *n.* [Gr. *mnēmē*, memory, and *technē*, art.] mnemonics.

Mo, in chemistry, molybdenum.

-mō, in printing books, a suffix to the number designating one of the equal parts into which a sheet is divided, the size of a page varying with the size of the sheet folded; as, 12*mo*, 16*mo*.

mō'ā, *n.* [from native (Maori) name.] any of an extinct group of very large, flightless birds of New Zealand, related to the ostrich.

Mō'ab·īte, *n.* [Heb. *Mo'ābh*, Moab.] a native or inhabitant of Moab, an ancient kingdom east and south of the Dead Sea.
Moabite stone; a slab discovered in the country of the Moabites, dating from perhaps 900 B.C. and bearing an inscription in ancient Semitic characters.

Mō'ab·īte, *a.* of Moab or the Moabites.

Mō'ab·īt·ish, *a.* same as *Moabite*.

mōan, *n.* [ME. *mone*; prob. from base of AS. *mænen*, to complain.]
1. a complaint; lamentation. [Rare.]
2. audible expression of sorrow or suffering; grief expressed in words or cries.
Sullen *moans*, hollow groans. —Pope.
3. any sound like this.

mōan, *v.i.* 1. to grieve; to make lamentations.
Unpitied and unheard, where misery *moans*. —Thomson.
2. to utter a moan or moans; as, the sea *moans*.

mōan, *v.t.*; 1. to lament; to deplore; to bewail audibly.
Ye floods, ye woods, ye echoes, *moan*
My dear Colombo dead and gone.—Prior.
2. to distress; to afflict. [Obs.]
3. to say with a moan.

mōan'fụl, *a.* sorrowful; expressing sorrow.

mōan'fụl·ly, *adv.* with lamentation.

mōat, *n.* [OFr. *mote*, from LL. *mota*, a mound, hill on which a castle is built, a castle, dike.]
1. a ditch or deep trench round the rampart of a castle or other fortified place, sometimes filled with water, for protection against invasion.
2. a pond; a lake. [Obs.]

mōat, *v.t.*; moated, *pt., pp.*; moating, *ppr.* to surround with a ditch for defense; as, a *moated* castle.

mob, *n.* [from L. *mobile vulgus*, the fickle crowd.]
1. a crowd or multitude of people, rude, tumultuous, and disorderly; also, any crowd.
2. a disorderly assembly.
Had every Athenian citizen been a Socrates, every Athenian assembly would still have been a *mob*. —Madison.
3. the masses; common people collectively: a contemptuous term.
4. a gang of criminals. [Slang.]
5. in Australia, a flock or herd, as of ducks, cattle, etc.
Syn.—populace, rabble, canaille.

mob, *v.t.*; mobbed, *pt., pp.*; mobbing, *ppr.* 1. to crowd around and attack.
2. to crowd around and jostle, annoy, etc., as in curiosity or anger.

mob, *v.t.* 1. to wrap in a cowl or veil so as to conceal the face, as with a cap. [Obs.]
2. to dress in an awkward manner. [Obs.]

mob'bish, *a.* like a mob; lawless and tumultuous; vulgar.

mob'by, *n.* fruit juice used in making brandy; also, the brandy after distillation.

mob'çap, *n.* [M.D. *mop*, a woman's cap.] formerly, a woman's cap or headdress, usually having broad bands to tie under the chin: also *mob*.

mō'bile (*or* -bēl), *a.* [L. *mobilis*, movable, from *movere*, to move.]
1. movable; not firm, stationary, or fixed.

Fifth Schedule

1-10μg HCV NC/ PBS 1X IN

2-5μg HBsAg/ PBS 1X IN

3-10μg HBsAg/ 10μg HCV NC / PBS 1X IN

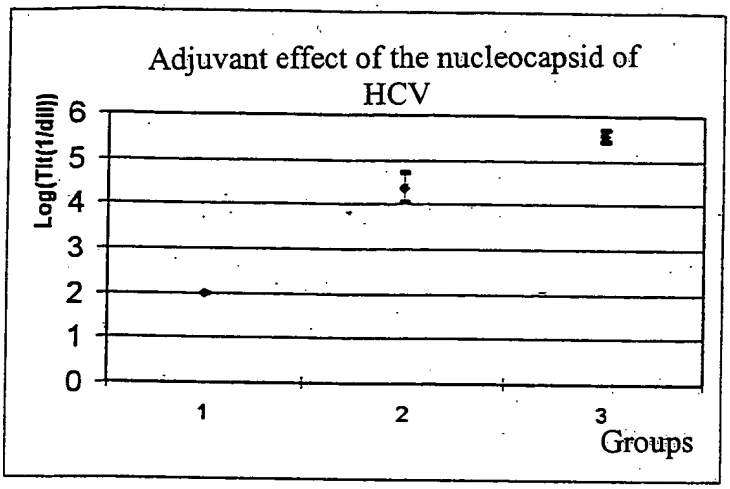


Fig. 5

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	J.C. Aguilar Rubido, et al.	Examiner:	Shanon A. Foley
Serial No.:	09/857,402	Group Art Unit:	1648
Confirmation No.:	3056	Docket:	976-11 PCT/US
Filed:	June 1, 2001	Dated:	December 2, 2002
For:	PREPARATIONS CONTAINING VIRUS-LIKE PARTICLES AS IMMUNOPOTENTIATORS ADMINISTERED THROUGH THE MUCOSA		

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. §1.132

Sir,

I, Julio César Aguilar Rubido, of Havana, Cuba, do hereby declare and state as follows:

1. I am a co-inventor named in U.S. patent application serial number 09/857,402 filed on June 1, 2001.

2. I hold a BSc degree in Biochemistry from Havana University.

3. I am employed as researcher by The Center for Genetic Engineering and Biotechnology, Havana, Cuba, the assignee of the above-referenced patent application.

4. I have worked in the field of vaccine and immunology research for 7 years.

5. The experiments described in Exhibit 1 were done by me or by persons directly under my supervision and control.

6. In the experiment entitled "A-Study of the capacity to induce gamma IFN (IFNγ) secreting cells by ELISPOT assay"described at page 1 of Exhibit 1, the highest response

was obtained in the group immunized with the nasal formulation HBsAg+HBcAg (fig 1). The results obtained demonstrated the capacity of the nasal route to induce strong gamma-interferon (IFNγ) secretion in spleen cells, inducing even better responses than the control group of parenterally administered alum-based vaccine.

7. Further, the enhancement of the immune response against HBsAg after coadministration of HBsAg and HBcAg was shown, evidencing the capacity of HBcAg to improve the cellular response against HBsAg administered alone in PBS. These results have therapeutic significance due to the involvement of IFNγ secretion by T-cells in HBV clearance. These results are consistent with the higher IgG2a response for HBsAg mixed with HBcAg compared with the above mentioned controls.

8. The results of experiments described at pages 2-3 of Exhibit 1 entitled "B-Study of the lymphoproliferative response of spleen cells by LPA" demonstrated the superiority of the nasal formulation HBsAg+HBcAg in terms of lymphoproliferative response specific for HBsAg. These results are highly significant for the design of therapeutic strategies taking into consideration the fact that a strong lymphoproliferative response correlates with a better course of the infection by HBV. These results show an improvement of the immune response after therapeutic administration of these major HBV antigens in the HBsAg + HBcAg formulation compared to the alum based vaccine.

9. Furthermore, at the cellular level, the combination of both antigens potentiates the immune response against both antigens, evidencing the synergistic interaction between both antigens (figure 2B). The other formulations of HBsAg and nucleocapsid antigens also had a similar behavior (also shown in figure 2B).

10. The immune tolerance against HBsAg in transgenic mice was abrogated by the administration of five (5) doses of the HBcAg+HBsAg formulation, and correlated with the disappearance of the HBsAg from the blood. The other formulations based in the

mixture of HBsAg and other VLPs: HBsAg+HCcAg or HBsAg+VLP of HPV, also generated the same effect in this animal model.

11. These results described in paragraph 10 was in contrast to the result obtained with the commercial vaccine, Engerix B, where the HBsAg is absorbed to alum salts and inoculated parenterally by the intraperitoneal route or compared to the use of HBsAg alone. The results are summarized in table 1 of Exhibit 1.

12. The results described in Example 2 of Exhibit 1 clearly demonstrate the enhancement of the immune response due to the new formulations for nasal administration of HBsAg in solution, coadministered with a VLP, showing an enhancing capacity and generating new properties to the resulting immune response. The resulting response against all the VLPs when mixed with HBsAg was strongly enhanced as compared to the VLP alone. This demonstrates the crossed effect in enhancing capacity of these antigens. This result in transgenic mice is consistent with the immune response observed in normal mice.

13. The immune tolerance against HBsAg in transgenic mice was abrogated by the administration of five (5) doses of the HBcAg+HBsAg formulation, and correlated with the disappearance of the HBsAg from the blood. The other formulations of mixtures of HBsAg and other VLPs: HBsAg+HCcAg or HBsAg+VLP of HPV, also generated the same effect in this animal model. This result was in contrast to the result obtained with the commercial vaccine, Engerix B, where the HBsAg is absorbed to alum salts and inoculated parenterally by the intraperitoneal route or compared to the use of HBsAg alone. The results are summarized in table 1.

14. In my professional opinion the chimpanzee is not the only model for human viral disease due to HCV or any other chronic disease. Normal and transgenic mice have are useful models to test the goal of therapeutic immunization before administration to humans.

15. One of the main characteristics of the human chronic disease is their specie-specificity. Some infections do not develop all the phases found in humans and in some other cases,

the disease is not reproduced in the same intensity as in humans. The above-described experiments show that it is possible to overcome the tolerance with the claimed formulations.

16. Our studies in humans are now in the first stages for prevention and treatment of chronic hepatitis B and are based in the already explained results in our animal models, hence we don't need to use chimps.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Dated: 4/12/2002

Signed: _____
Julio César Aguilar Rubido

EXHIBIT 1

Example 1

The interaction of a nucleocapsid antigen (HBcAg) and the HBsAg induced an improved response against both antigens and also against HBcAg evidencing a synergistic response at the cellular level.

A- Study of the capacity to induce gamma IFN secreting cells by ELISPOT assay.

The cellular immune response generated by the nasal formulation of HBsAg and HBcAg, groups of eight weeks old 8 to 10 female balb/c mice were immunised three times at doses of 5μg of HBsAg per mice. Innoculations were carried out times every 2 weeks with the formulations presented in table 1. The immune response was determined two weeks after the last administration.

The ELISPOT assay was performed as described, briefly, lymphocytes from spleens were stimulated with the peptide $HBsAg_{(28-39)}$ presented by the murine tumor cells p815 that were used as antigen presenting cells. Spleen cells were isolated after surgical excission, treatment with ammonium chloride as erythrocyte lysis solution and then washed three times.

After washing steps, the cells were counted and distributed at $2x10^6$ cells per milliliter in 10 mL RPMI fetal calf serum in $25cm^2$ flasks (Nunc), and stimulated with 10 μg/mL of peptide $HBsAg_{(28-39)}$. After culturing during four days, in CO_2 5%, half of the total medium was substituted and new media containing $2x10^4$ U/mL of IL2 was added. On day seven, cells were collected and counted. Subsequently, 10^4 and $5x10^4$ cells per well were added to 10^5 p815 cells, previously pulsed with the peptide for one hour. Stimulation with 2μg per well concanavalin A was used as a positive control of the assay. Every group was controlled by the same number of wells incubated with non-pulsed p815 cells as a negative control.

As a result of this experiment, the highest response was obtained in the group immunized with the nasal formulation HBsAg + HBcAg (fig 1). The results obtained demonstrated the capacity of the nasal route to induce strong gamma-interferon (γIFN) secretion in spleen cells, inducing even better responses than the control group of parenterally administered alum-based

vaccine. Also the enhancement of the immune response against HBsAg after coadministration of HBsAg and HBcAg was shown, evidencing the capacity of HBcAg to improve the cellular response against HBsAg administered alone in PBS. These results have therapeutic significance due to the involvement of T-cell gamma interferon secretion in HBV clearance. These results are consistent with the higher IgG2a response for HBsAg mixed with HBcAg compared with the above mentioned controls.

Fig 1. Results of the ELISPOT experiment to determine the capacity of gamma IFN secretion by spleen lymphocytes. The results of the experiment are expressed in cells per million.

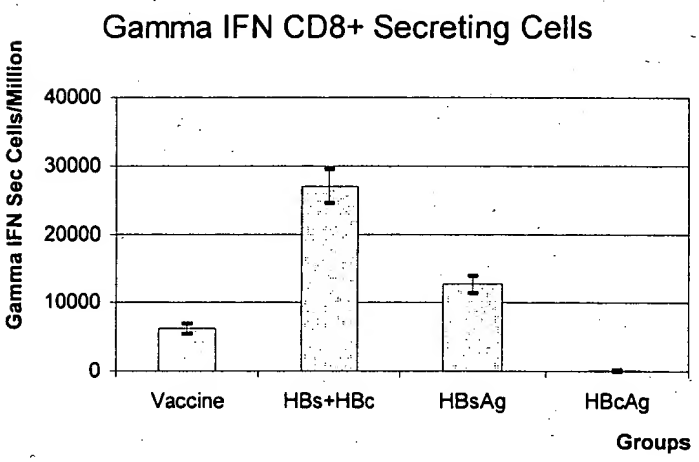


Table 1

Groups	route
G1- 5μg HBsAg / alum	IM
G2- 5μg HBsAg / 5μg HBcAg	IN
G3- 5μg HBsAg / PBS 1X (*)	IN
G4- 5μg HBcAg / PBS 1X (*)	IN
G5- Non Immunised group	

B- Study of the lymphoproliferative response in spleen cells by LPA assay.

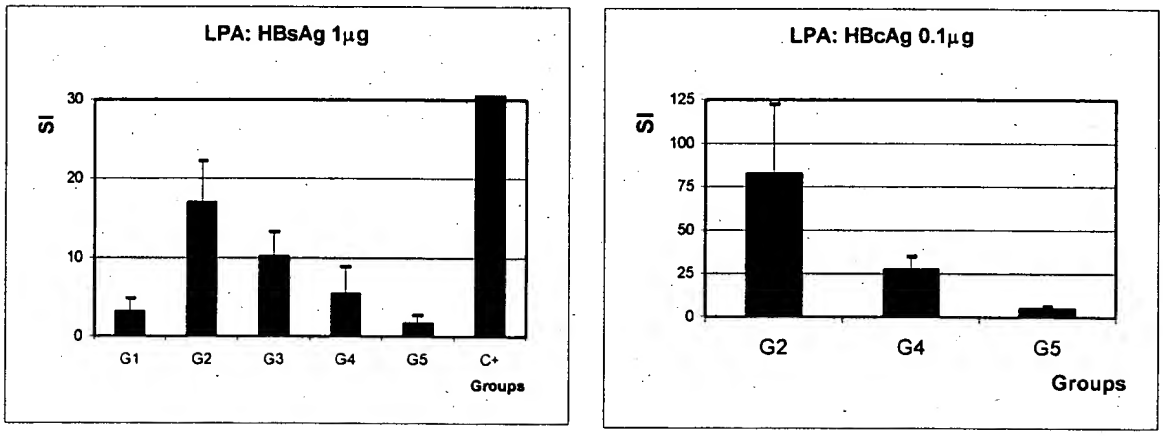
Cells of the animals immunized with the formulation HBsAg + HBcAg described above, and respective controls, were incubated in 5% CO_2 at 37 degrees Celsius in culture plates of 96 wells, in presence of HBsAg in concentrations of 1 and 0.1 μg/mL, during 4 days. Then, cells were pulsed with H^3 thymidine at 1 μCi per well. Cells were cultured by 12 hours and then were harvested and counted with a scintillation counter at 1-minute intervals per assay per well.

The results corresponding to the stimulation index (SI) of the lymphoproliferative response in the immunization conditions previously described are presented in the figure 2A.

These results demonstrated the superiority of the nasal formulation HBsAg + HBcAg in terms of lymphoproliferative response specific for HBsAg.

This results also has significance in the design of therapeutic strategies taking into consideration that a strong lymphoproliferative responses correlates with a better course of the infection by HBV, suggesting a potential improvement of the immune response after therapeutic administration of these major HBV antigens in the HBsAg + HBcAg formulation compared to the alum based vaccine. Also, at the cellular level, the combination of both antigens potentiates the immune response against both antigens, evidencing the synergistic interaction between both antigens (figure 2B). The other formulations of HBsAg and nucleocapsid antigens also had a similar behavior (figure 2B).

Fig 2. Results of the LPA assay. The results of the LPA experiment represent the stimulation index of wells incubated with (A) HBsAg 1μg/mL and (B) HBcAg 0.1μg/mL and their standard deviation.



Example 2

Overcoming the tolerance state in transgenic mice.

An immunization schedule with the formulations containing HBsAg and different nucleocapsid antigens (HBcAg, HPV VLPs, HCcAg and GAG) was carried out in six groups of five BALB/C transgenic mice carrying the HBV antigens with an average of 15 μg/mL in sera. These mice have demonstrated to be tolerant to the HBsAg and HBeAg at the cellular and humoral levels.

The immune tolerance against HBsAg in transgenic mice was abrogated by the administration of 5 doses of the HBcAg + HBsAg formulation, and correlated with the disappearance of the HBsAg from the blood. The other formulations based in the mixture of HBsAg and other VLPs: HBsAg + HCcAg or HBsAg + VLP of HPV, also generated the same effect in this animal model. This result was in contrast to the result obtained with the commercial vaccine, Engerix B, where the HBsAg is absorbed to alum salts and inoculated parenterally by the intraperitoneal route or compared to the use of HBsAg alone. The results are summarized in table 1.

The result described in this example clearly demonstrated the enhancement of the immune response due to the new formulations for nasal administration of HBsAg in solution, coadministered with other VLP, showing an enhancing capacity and generating new properties to the resulting immune response. The resulting response against all the VLPs joined to HBsAg was strongly enhanced as compared to the VLP alone. This demonstrates the crossed effect in enhancing capacity of these antigens. This result is consistent with the immune response observed in normal mice.

Table 1. HBsAg secreting HBV tg mice at day 100 after five doses of 5 mcg of both antigens.
* mice clearing the HBsAg from blood developed strong lymphoproliferative (LPA) response.

Antigen Formulation	HBsAg in sera	LPA anti HBsAg over 5 of Stim.Index
HBsAg + HBcAg (A)	0/5	5/5
+ HCcAg	0/5	5/5
+ VLP HPV	1/5	4/5
+ GAG	0/5	5/5
HBsAg alone	4/5	1/5*
Engerix B	5/5	0/5
Non treated tg mice	5/5	0/5
Non tg mice + Engerix B	0/5	5/5

The levels of IgG titers specific for HbsAg developed by HBsAg transgenic (Tg) mice were statistically superior ($p<0.05$) to those generated in Tg mice after the administration of the commercial vaccine. As shown in table 1, the cellular response was higher for those mice clearing the HBsAg from the blood as was also evidenced in the mouse that cleared the HBsAg in the group immunized with HBsAg alone.

It is important to point out that the effect observed for groups containing HBsAg and a viral nucleocapsid were not observed for the groups immunized with the respective nucleocapsid alone. This excludes the possibility of a non-specific response caused by nucleocapsid antigens. The already explained superiority in mucosal and cellular responses was also obtained for transgenic mice, in those groups immunized with the formulations based in HBsAg and different

nucleocapsid antigens compared to the group of Tg mice immunized with the commercial vaccine as a further support of the therapeutic use of the formulations of HBsAg and nucleocapsid antigens in the treatment of the HBV chronic infection.

Example 3

Tolerance induced by injection and oral feeding

Preliminary results to explore the subversion of tolerance state to HCV core protein, HPV E1-E2 protein and the HIV gag protein in transgenic mice expressing the corresponding antigen in sera have evidenced the capacity of this kind of formulations to overcome the normal state of non responsiveness to the antigens expressed found in those Tg mice at the cellular levels. In the case of these antigens, our experiments have shown the abrogation of tolerance induced by peritoneal injection of high doses and the feeding of a low amount of antigens for more than a month.

These specific models where the tolerance state was obtained by the injection of high amounts of the corresponding antigens along with antigen feeding, demonstrated the capacity of the described formulations nasally administered to subvert the tolerant state to the particular antigens. Conversely, it was impossible to change this state in mice injected parenterally with the same antigens in alum. The immune response was controlled by the induction of proliferative activity in individual mice to the antigens used to tolerize them. All mice treated with the nasal formulations of HbsAg and the homologous antigen (the antigen used to tolerize) induced a stimulation index superior to five (5), while control mice immunized with the homologous antigen in alum did not proliferate sufficiently to be considered clearly positive.

This kind of animal model and treatments are also used in the simulation of chronic diseases like autoimmune processes. Our preliminary results with the model based in transgenic mice to simulate the tolerance induction during chronic diseases point to similar results to those obtained in example 2.